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Effect of subtransition in 1,2-dipalmitoylphosphatidylcholine model membrane on the spin probe motion

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The effect of subgel → gel phase transition (subtransition) on conventional electron spin resonance spectra of cholestane, fatty acid and alkylammonium-type spin probes has been studied in aqueous 1,2-dipalmitoyl-*sn*-phosphatidylcholine dispersions. The cooperative onset of the cholestane spin probe rotation about its long axis, with an effective correlation time of 2–3 ns, has been detected at a temperature coinciding with the calorimetric substransition, indicating onset of the host lipid rotational motion. The lipid rotation results in dissolution of the spin probe clusters in the host lipid. In the gel phase, the lateral distribution of impurity molecules is more isotropic than in the subgel phase.

Dipalmitoyl-*sn*-phosphatidylcholine, 1,2-; Subtransition; ESR; Spin probe

1. INTRODUCTION

1,2-Diacylphosphatidylcholine (PCs) in excess water are extensively studied as models of the lipid part of biological membranes. Their phase behaviour in excess water can be described in the form $L_{c,} \rightleftharpoons L_{\beta,} \rightleftharpoons P_\beta \rightleftharpoons L_\alpha$, where the three solid-like phases $L_{c,}$, $L_{\beta,}$, and P_β have extended acyl chains predominantly in the *trans* configuration, while the lamellar fluid phase L_α has disordered acyl chains due to *trans-gauche* isomerization (see [1–6] for details and references). In the $L_{\beta,}$ and $L_{c,}$ phases, the acyl chains are tilted to the bilayer normal, but become perpendicular to the bilayer plane in the P_β phase; the lamellar surface of the P_β phase is rippled [7–11]. The $L_{\beta,} \longrightarrow P_\beta$ phase transition is called 'pretransition', $P_\beta \longrightarrow L_\alpha$, the 'main transition', and $L_{c,} \longrightarrow L_{\beta,}$, the 'subtransition'. The subtransition is exclusively slowly reversible upon cooling and heating compared to the other phase transitions in PCs. Conversion $L_{\beta,} \longrightarrow L_{c,}$ occurs only if the PC-H_2O system has been annealed at low temperatures for an extended period of time [12–15].

In the present communication, we study effects of subtransition on spectral parameters of spin probes located in the hydrophobic or polar part of the 1,2-dipalmitoyl-*sn*-PC (L-α-DPPC) bilayer.

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2. MATERIALS AND METHODS

L-α-DPPC was purchased from Fluka (Buchs, Switzerland), 4-(*N*-hexadecyldimethylammonium)-2,2,6,6-tetramethylpiperidinyl-oxyl bromide (CAT-16) spin probe was from Technika (Sofia, Bulgaria), and 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyl-oxyl (16-DSA) and 4′,4′-dimethylspiro[5α-cholestane-3,2′-odazolidin]-3′-yloxyl (CSL) spin probes were from Syva (Palo Alto, USA).

L-α-DPPC and CSL spin probe were mixed in a molar ratio of 100:1 or less in chloroform. Solvent was evaporated under stream of nitrogen gas followed by a diffusion pump evacuation. The lipid was dispersed in the distilled water in a weight ratio of 1:25 and incubated at 45°C for 1 h. During this incubation the dispersion was mixed by vortexing several times for about 1 min at the elevated temperature. The dispersion was then transferred into a glass capillary and centrifuged on a haematocrit centrifuge for 15 min. The supernatant was discarded and hydrated lipid sealed in the capillary. Samples with 16-DSA and CAT-16 spin probes were prepared as described above, except that the labeling step was performed after

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preparing the L-α-DPPC dispersion in water. The spin probe was deposited onto a plastic tube wall from ethanol and solubilized thereafter by the lipid dispersion using the vortex mixing and short time sonication in a bath ultrasonicator at temperatures about 45°C. The molar ratio of probe to lipid was 1:100 or less.

Sample capillaries were contained in glass tubes filled with silicon oil for thermal stability. Samples were annealed at 0°C for various periods of time before being loaded into precooled cavity of the ESR spectrometer. In the first heating scan the samples were heated from 0°C to 24–30°C. The second heating scan followed after recooling the sample in the cavity to 0°C for 30 min. The heating was performed in 2°C steps. The ESR spectrum was recorded after equilibration of the sample for 5 min after each temperature step.

Spectra were taken by an ERS 230 X-band spectrometer (ZWG AdW DDR, Berlin, GDR) using the 100 kHz modulation technique. Typical instrumental settings were: up to 7 mW microwave power (depending on the spectrum appearance), modulation amplitude less than 0.2 mT, scan rate 1.5 mT/min or less, accuracy of temperature settings ± 0.5°C. The value of the z component of the spin probe nitrogen hyperfine splitting tensor A_{zz} was determined from the rigid limit powder pattern spectra recorded at the liquid nitrogen temperature.

3. RESULTS AND DISCUSSION

The 16-DSA and CSL spin probes in L-α-DPPC dispersions annealed at 0°C for a prolonged period (up to 96 h) display powder pattern triplet spectra superposed on a broad singlet line. This broad line disappears in the first heating scan at about 18°C and does not occur in the second heating scan after recooling the sample at 0°C for 30 min. It was not discernible in samples with the CAT-16 spin probe. The broad line can be ascribed to probe molecules in sites in the L-α-DPPC lattice with high probe concentration where the nitrogen hyperfine triplet collapses to an exchange narrowed Lorentzian singlet due to strong exchange interaction [16,17]. Most probably, fractions of the spin probes form clusters in the L_{c_1} lattice defects during annealing of samples at 0°C: these clusters dissolve in the host lipid at about 18°C. This temperature coincides with the subtransition temperature observed calorimetrically in L-α-DPPC dispersion annealed and scanned under conditions close to ours [6,12,18–21].

Spectral parameters of the CSL nitrogen hyperfine triplet change with the increase of temperature and differ in the first and second heating scan within the region from 0°C to 16–18°C. In the lipid bilayers, the CSL spin probe is oriented with its long axis preferentially perpendicular to the plane of the bilayer and thus the principal x and z axes of the hyperfine splitting tensor **A** lie approximately within the bilayer plane. Since $A_{zz}:A_{xx} \approx 5–6$, the powder pattern ESR spectra of the CSL probe are sensitive to the probe rotation about its long molecular axis. For situations in which the powder pattern spectra appear similar to the rigid limit spectra, the correlation time of the CSL probe rotation about its long axis, $\tau_{\parallel}$, can be estimated from the empirical equation:

$$\tau_{\parallel} = a(1 - A_{max}:A_{zz})^b \quad (1)$$

where A_{max} is one-half of the separation of the outer spectrum extrema, A_{zz} is the z component of the CSL nitrogen **A** tensor, and $a = 2.596 \times 10^{-10}$ s and $b = -1.396$ are calibration constants obtained from spectra simulations and empirical calibrations [22,23]. This equation is valid for $\tau_{\parallel}$ in the range from 2×10^{-9} to 7.5×10^{-8} s. The values of $\tau_{\parallel}$ at selected temperatures deduced from the spectra are shown in table 1 and compared with those obtained in [24].

It is seen that reliable values of $\tau_{\parallel}$ can be obtained from spectra measured up to 24°C. The values of $\tau_{\parallel}$ below 18°C are dependent on the annealing conditions, while above 18°C they are independent of both annealing temperature and time, and coincide with the values obtained in [24]. The Arrhenius plot of data in fig.1 clearly demonstrates that the temperature dependence of $\tau_{\parallel}$ is different for annealed and nonannealed samples. In the nonannealed sample $\tau_{\parallel}$ decreases monotonously with the increase of temperature from 4°C to

Table 1

Rotational correlation time $\tau_{\parallel}$ of the CSL spin probe in L-α-DPPC dispersions

t (°C)	$\tau_{\parallel}$ (ns)			
	A	B	C	D
4	9 ± 1	>10		14 ± 5
10	6 ± 1		16 ± 5	9 ± 2
14	3.6 ± 0.3	4		8.3 ± 0.2
20	2.4 ± 0.2		2.6 ± 0.2	2.4 ± 0.2
24	1.8 ± 0.1	1.9		1.9 ± 0.2
34	1.15 ± 0.04	1.2	1.19 ± 0.04	

A, 30 min at 0°C, this work; B, overnight annealing at 4°C, [24]; C, 17 h annealing at 0°C, this work; D, 96 h annealing at 0°C, this work

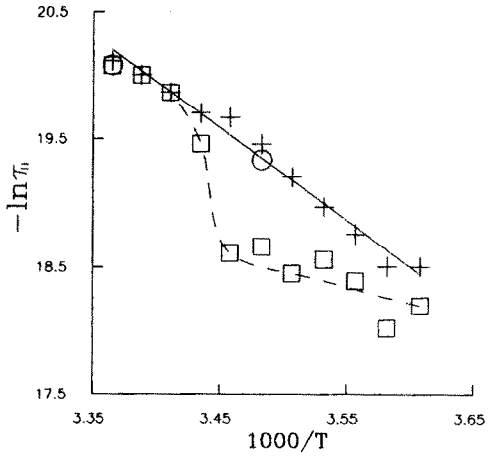

Fig.1. Temperature dependence of the correlation time $\tau_\perp$ (in s) of the CSL spin probe. T is expressed in K. (+) 30 min at 0°C, this work; (○) overnight annealing at 4°C, [24]; (□) 96 h annealing at 0°C, this work.

24°C. The temperature dependence of the annealed sample indicates the onset of the CSL rotation about its long axis between 16°C and 18°C. This correlates well with the existence of the calorimetric subtransition which was observed at about 17–18°C [6,12,18–21]. The onset of rapid rotational motion is also consistent with other known changes in L-α-DPPC properties at the subtransition – it is accompanied by a decrease in the lateral packing density of lipids [11,18,20] and cooperative increase of lipid specific volume [14,15], by an increase in both the lipid hydration [11,13,18] and the rate of the lipid head group motion about bilayer normal [20]. ^{1}H NMR studies have detected an increase in the proton spin-lattice relaxation time [25] and proton second moments [25,26] in the annealed samples of L-α-DPPC dispersions. Second moments drop sharply at the subtransition and this effect has been interpreted as the onset of restricted lipid chain rotation in the $L_{\beta'}$ phase (correlated rotational 90° jumps between the two preferential orthorhombic chain positions) [26]. ^{2}H NMR studies of acyl chain perdeuterated L-α-DPPC have also detected that in $L_{\beta'}$ phase most of the lipid molecules rotate about their long axes [27]. Our results are not only consistent with these NMR observations, but also clearly demonstrate that above the subtransition the rotational motion is observable on the ESR time scale.

The differences in the 16-DSA and CAT-16 spectral parameter A_{max} in the $L_{c'}$ and $L_{\beta'}$ phases were rather small and nonimpressive. This is understandable, because the principal x and y axes of the hyperfine splitting tensors lie within the bilayer plane and $A_{xx} \approx A_{yy}$ [22,28], so that the long axis rotation in the slow motional region (2 ns $\leq \tau_\parallel \leq$ 75 ns) should not affect the value of A_{max} deduced from the powder pattern spectra appreciably. However, we have observed small but significant and reproducible differences in the values of linewidth parameter Δ_1 (outer half-width at half-height of the low-field extremum) in the annealed and nonannealed samples. As an example, the Arrhenius plot of Δ_1 of the 16-DSA spin probe is shown in fig.2. It is seen that between 2°C and 16°C, Δ_1 goes through a weak and broad maximum and that the values of Δ_1 are higher in the annealed sample. Similar effects have been observed also for the CAT-16 and 16-DSA spin probes (not shown). Above 18°C, the values of Δ_1 in annealed and nonannealed samples are virtually the same. Several factors affect the value of Δ_1 in the slow motional region powder pattern spectrum – unresolved proton hyperfine structure, lifetime broadening competing with the motional averaging, heterogeneity of the spin probe environment and spin-spin exchange interaction [29]. Our results demonstrate that the sum of different contributions to the linewidth parameter Δ_1 is different

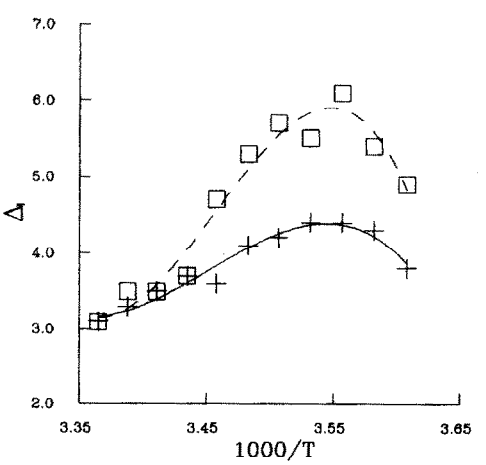

Fig.2. Temperature dependence of the linewidth parameter (outer half-width at half-height of the low-field extremum) of the 16-DSA spin probe. T is expressed in K and Δ_1 is expressed in Gauss. For symbols see fig.1.

for annealed and nonannealed samples, and that this difference disappears at the subtransition. Though qualitatively, this observation might indicate more isotropic lateral distribution of spin probes (= impurity molecules) in the L_{β} phase.

In conclusion, we have observed the onset of the lipid long axis rotation at the $L_{c,} \longrightarrow L_{\beta,}$ phase transition accompanied by the dissolution of the spin probe clusters in the host lipid lattice and resulting in the more isotropic lateral distribution of the spin probe molecules in the $L_{\beta,}$ phase.

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